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VARIATIONS IN THE POLLEN GRAIN OF *PICEA EXCELSA*[1]

JAMES B. POLLOCK

INTRODUCTION

WHILE teaching the reproduction of gymnosperms to a class in the University of Michigan, the writer observed a very unusual structure in a pollen grain of *Picea excelsa* L. (Pl. 1, Fig. 10). The structure of this pollen grain was reported at the meeting of the Botanists of the Central States, in December, 1901, and the suggestion was made that possibly the extra cells found at the dorsal side of the pollen grain should be interpreted as a prothallium, much more highly developed than usual.

On using some of the same material for other classes it was found that the structure in question occurred rather frequently, along with other variations in the structure of the pollen grain of Picea, so that it was considered of sufficient importance to report in the present paper. As will appear later, a further examination of numerous pollen grains has led to an interpretation of the observation different from the one suggested above.

The material was a part of the stock material of the botanical laboratory at the University of Michigan, collected May 14, 1884, but there was no indication on the label as to who was the collector, where it was collected, or of the method of killing the material. The condition of the material suggested that it was killed by putting it directly into alcohol. In all the grains the protoplasm was considerably contracted from the walls of the cells, but was otherwise well preserved.

METHODS

In order to make the interior structure of the pollen grains as plain as possible, the anthers, which were almost at the stage of dehiscence, were allowed to stand in a solution of Kleinenberg's hæmatoxylin for twenty-four hours. They were then washed in alcohol and changed very gradually from 96% alcohol to pure clove oil. In the clove oil the pollen sacs were broken up and the pollen grains set free in the oil. The pollen grains were studied by mounting a drop of the oil containing them. It was found that the pollen grains stained very unequally, some not having stained at all, and others having stained so densely that the interior could not be seen. Examination showed that the unstained ones were often the most satisfactory objects for study, so no further efforts at staining were made. This is the method used in demonstrating the interior structure of the pollen grain to large classes, and the author has no claim as the originator of the method. When the grain is thoroughly permeated by the clove oil it becomes so transparent that the interior can be seen very plainly.

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[1] Contribution 88 from the Botanical Department of the University of Michigan.

The contracted condition of the protoplasm of the various cells in the interior of the pollen grain made the outlines of these cells very plain, and their number easy to determine with certainty in a large series of the grains examined. Thus the crudeness of the method of killing the material proved a decided advantage in the study of it. Another advantage appeared in mounting the pollen grains in clove oil, in that the same individual pollen grain could be studied from different points of view on causing it to roll over by means of pressure on the cover glass. Two views of the same pollen grain are shown in Figs. 11 and 12, also in Figs. 13 and 14 (Pl. 1).

Historical

The older literature concerning the pollen grain has reference to the external characters and structure, and for the internal structure there is no need to go farther back than 1834, when von Mohl published his work, *Ueber den Bau und die Formen der Pollenkörner* ('34). In this article von Mohl cites the older literature on the subject, and figures pollen grains of a few gymnosperms. His figures, however, do not show internal structure, and only in the work of Fritzsche (1836), do we first find figures which show something of the internal structure of the pollen of gymnosperms as we now know it.

Among numerous species of angiosperms and a few gymnosperms Fritzsche ('36, p. 693) described and figured the pollen grains of *Pinus sylvestris* and *Pinus larix* (*Larix europæa*). The terms he used for the different cells formed in the pollen grains were totally different from those now in use. He did not regard the bodies seen as cells, nor did he have any knowledge of their function or homology. His figures show, however, a remarkable accuracy of observation, if we take into consideration the state of knowledge of his time, and the comparatively poor microscopes and crude microscopical methods. He examined the pollen grains in oil of lemon (Citronenöl), and he undoubtedly saw one of the disintegrating prothallial cells in both *Pinus sylvestris* and *Larix europæa.* His Plate 3, Figure 10, shows this for the former, and Figure 14 for the latter. In Larix, besides the disintegrating prothallial cell, he showed the cells which were later called the "stalk" and "body" cells. The latter, Fritzsche called the central vesicle, and the other two cells he called "Zwischenkörper."

Later investigations have shown that in Larix, and sometimes in Pinus also, there are two prothallial cells cut off which later may disintegrate wholly or partly. It is not safe, however, to conclude that these genera vary in the number of prothallial cells formed merely because Fritzsche did not see two of them while other writers have done so. When we remember the difficulties encountered by later observers equipped with much better microscopes than Fritzsche could have had in 1836, the wonder is that he saw so much.

Meyen ('39) pointed out that Fritzsche's "Zwischenkörper" were really cells, and that one of them served as stalk of attachment to the larger cell. This was probably the first application of the term "stalk" to this cell ('39, p. 189).

For about two decades after this time the literature concerning the pollen grain is occupied mostly with evidence for and against the view of Schleiden, advanced in 1837, that the embryo has its origin in the pollen tube. Schacht ('52, p. 407) went so far as to say that the pollen is the egg of the plants, and that there is no real analogy between animal and plant fertilization among phanerogams.

Hugo von Mohl ('55), Hofmeister ('55), and others opposed these views, and it was only when Schacht himself discovered in *Gladiolus segetum* ('58a) a case in which the embryo undoubtedly originated in a cell which was in the embryo-sac previous to the advent of the pollen tube, that the controversy practically came to an end in favor of the views of Hofmeister and von Mohl.

Schacht ('60) reported variations in the structure of the pollen grains of several gymnosperms. On page 143 of the article designated he says that the pollen of *Cupressus sempervirens* has a slightly elongated roundish form, which has divided into two unequal cells when the anther opens. *Thuja orientalis* shows the same relation, only here *oftener than in Cupressus* (italics are mine) further division takes place in the larger cell. Two of these extra divisions are shown in his Figures 22 and 23, Plate 17. The text cited above implies that similar divisions are formed in Cupressus, but none are figured. Schacht also found variations in the number of cells in pollen grains of *Larix europœa,* shown in his Figures 4 and 5. Since Schacht did not understand that one or more of the cells first cut off in these pollen grains may disintegrate, it is possible that his observations do not really mean a variation in the total number of cells formed in Larix, but his figures seem to the writer to indicate that he actually observed such a variation in *Larix europœa.* He also states ('60, p. 144) that *Abies pectinata* (Tanne), *Picea vulgaris* (Fichte) [*Picea excelsa* L.], and *Pinus sylvestris* (Kiefer) are like Larix in the interior of the pollen grain, only in the first two there are almost always three cells, and in the latter sometimes only two. No figures are given for these, but it seems probable that Schacht really observed variations in the number of cells in the pollen of the species named. Schacht was probably the first to figure the sperm cells ('58a) though he did not understand their function.

Hofmeister ('62, p. 406) described the structure in the pollen of Juniperus, Taxus, and Thuja differently from what it is now understood to be, but the apparent variation is probably due to a wrong interpretation.

Juranyi in a preliminary notice in 1870 and in the fuller article in 1872, reported a variation in the number of cells found in the pollen of *Ceratozamia longifolia* Miq. He found that the one-celled stage of the pollen grain divided into a large and a small cell, the latter bulging into the former. The smaller cell divides again into two unequal cells, the smaller lying against the pollen grain wall, the larger one being hemispherical. Most pollen grains are ripe at this stage of development, and undergo no further changes till the time of fertilization. Often, however, one meets a pollen grain which does not remain at this stage of development, but goes a step farther in that the hemispherical cell divides once more, so that a three-celled body is found in the interior of the pollen grain. Juranyi's Figures 3 and 8 of Plate 33 ('72) show very well what he found. He, as well as Hofmeister, was mistaken as to the divisions of the smaller cells, since later investigations have shown that the small cells are cut off successively from the larger one and that only the last of the smaller cells divides when the "stalk" and "body or central" cells are formed. Juranyi's figure ('72, Pl. 33, Fig. 8), which shows three small cells, may either be an actual variation in the number of cells, or it may be a case of premature division into "stalk" and "central" cells. No certain conclusion can be reached, but if the division into "stalk" and "central" cells is the same in Ceratozamia as Webber found in Zamia, then Juranyi's figures of the former do indicate a variation in the number of prothallial cells or else a two-celled stalk. In 1882, Juranyi reported that he had seen a few cases among the Cycadaceæ in which the small cells could divide parallel to the long axis of the prothallium (Vorkeim), and that in *Larix europæa* he found some pollen grains in which the prothallium ended with two cells lying beside each other, and separated by a division wall. The upper cell had divided parallel to the long axis of the prothallium. In the cases just mentioned in which the small cells in some of the Cycadaceæ divide parallel to the long axis of the prothallium, it cannot be questioned that we have a case of variation from the normal, both as to the number of cells formed, and as to the relation of those cells to each other in space. In the cases of *Larix europæa* in the upper cell there may have been an earlier division than usual into the two sperm cells, though at the time these observations were made the exact origin of the sperm cells was not understood.

Strasburger, in *Die Coniferen und Gnetaceen* ('72) added much to our knowledge of these plants, though some of his observations were inaccurate and his earlier conclusions wrong. Strasburger wrongly interpreted the disintegrating cells at the base of the pollen grain as mere slits in the wall having an origin similar to that of the wings of the grain ('72, p. 127). Schacht ('60) had interpreted these correctly as also had Meyen ('39). Following Hofmeister's suggestion Strasburger said that the cells formed in the interior of the pollen grain could only be considered vegetative cells, homologous to the prothallium of the fern. He supposed the large cell of the pollen grain corresponded to an antheridium, believing that it furnished the male gametes. He also thought that the cell structure was the same in Pinus, Podocarpus, and Cupressus ('72, p. 130). Later investigations have shown that no two of these are *exactly* alike. On page 131 Strasburger ('72) remarks that the number of cells which are formed by division in the pollen grain in Cycads and Coniferæ remains constant. It never exceeds two, according to his observations. The report of a larger number can only be founded on the view that the split in the wall is a disintegrated cell.

In a paper published in 1875, Tschistiakoff wrote that where there are several small cells, they arise *by successive division of the large pollen cell* as in Larix, or the second cell can divide to form two cells as in Ginkgo ('75, p. 100). In Pinus according to Tschistiakoff two, or at the most three small cells arise. The one or two first cells arise by a real division of the large pollen cell. The small cells may have their whole volume in the intine. The third large cell, arching into the pollen grain, arises by division of a small cell. In Abies the third cell forms by free cell formation, this divides into two, each of these can divide again, and these secondary cells can divide longitudinally or transversely. The small cells (Suspensorzellen) can likewise divide.

Though some of the observations made by Tschistiakoff were inaccurate, at least two new truths were presented, even if they were not proved, namely, that several small cells were cut off successively from the large pollen cell in such grains as those of Larix and Pinus, and that the last one of the row of cells formed, became the progenitor of spermatozoid mother cells. His article also mentions variations in the prothallium of Abies, and in the number of cells formed in Thuja, where he says there may be two divisions instead of one, and these may be in different directions.

Both of the two truths presented by Tschistiakoff were either denied or ignored by later writers, (Strasburger, '78; '80). While Juranyi ('82) admitted Tschistiakoff's view of the origin of the small cells, proving it in Cycads, and believing it true for Coniferæ, both Juranyi and Strasburger believed the large tube cell of the pollen grain furnished the male gametes and therefore represented an antheridium, and it was not until Belajeff ('91–'93) proved the contrary for *Taxus baccata* that the correct view was finally established, and generally accepted. Both Belajeff ('91–'93) and Strasburger ('92) showed that in the Abietineæ also it was not the tube cell, but the end cell of the row of interior cells formed, from which the male gametes were derived. Strasburger ('84) had previously admitted that Tschistiakoff's view of the origin of the small cells in the pollen was correct, and he had also shown that in *Larix europæa* the third small cell cut off from the large pollen cell divided into two cells which he named the "stalk" (see Meyen, '39, p. 189) and "body" cells respectively. The recognition of the "splits" in the wall of Larix pollen as disintegrating cells ('84, pp. 2–3) was a confirmation of the earlier views of Schacht and Meyen.

Strasburger ('92) reported some interesting variations in the number and arrangement of cells in the pollen of *Ginkgo biloba*. Ordinarily there are three narrow cells and one large one found in the ripe pollen grain. Usually the first two of the former are disorganized, but it often happens that two prothallium cells are absorbed and yet two permanent ones are found. Also pollen grains were repeatedly observed in which there were three permanent inner cells preceded by one absorbed cell. Finally one grain was seen in which all three prothallium cells were permanent, and the first of these was divided longitudinally. Also (Strasburger, '92, p. 18) the stalk cell of the antheridium appears to divide under some circumstances.

Since 1893 the most important step in the progress of our knowledge of the pollen of gymnosperms was the discovery of motile spermatozoids in 1896 and 1897, by Ikeno ('98) in Cycas, by Hirase ('97) in Ginkgo, and by Webber ('97) in Zamia. Webber's (:01) observations on Zamia and Ginkgo are interesting from the point of view of variation in those genera. He did not work out the details of the formation of prothallial cells, but in the species of Zamia studied there were always at least two prothallial cells cut off at one side and projecting into the grain as in Ceratozamia and Macrozamia, and different from Cycas and Ginkgo, where the walls are straight across the grain and not arching into it. It seemed to Webber, however, that three prothallial cells were occasionally formed, and in this case the first one was resorbed as described by Strasburger and others in Pinus, Ginkgo, etc., remaining as a dark refractive layer in the wall of the pollen grain, situated at the point of contact of the other cells. In many instances in mature grains, and in later stages, during germination, no indication of this resorbed prothallial cell can be observed, but in some cases it is unmistakable. A careful investigation of the development of the pollen in Zamia will have to be made before it can be determined whether three prothallial cells are regularly formed or whether the remnants of a third cell, occasionally observed, are to be considered as cases of rare and somewhat abnormal development.

Webber (:01, p. 24) clears up a point in the origin of the stalk cell and the central cell (body cell of Strasburger) showing that they originate by the division of the inner prothallial cell. Up to the time of Webber's full publication this point was quite obscure and this brings it in agreement with the facts in other gymnosperms investigated, except that the sterile cell bulges strongly into the stalk cell and this had led to a wrong interpretation. Webber also shows that Ginkgo is *probably* similar to Zamia on this point, and Seward and Gowan (:00, p. 130) show that this is actually the case. They also state that in Ginkgo the pollen grain at maturity contains a prothallium of from 3 to 5 cells.

Lang ('97) reports that *Stangeria paradoxa* has two prothallial cells when pollen is shed, but his Figure 18, Plate 22, seems to show three.

An interesting question is suggested by the different results observed in two species of the genus Ephedra. Jaccard ('93) reported that in *E. helvetica* at the time of anthesis there were in the pollen grain three free nuclei, but not separate cells, and he called these nuclei respectively the prothallium nucleus, the generative nucleus, and the tube nucleus. Land (:04) in *Ephedra* *trifurca* found two persistent prothallial cells, the first cut off by a wall, the second not so cut off, while a third cell which he calls the primary spermatogenous cell divides into the stalk and body cells, which are not separated by a wall, but have a common membrane (not cellulose but plasmic?). At time of anthesis therefore it has two prothallial cells, a stalk cell, a body cell, and a tube nucleus. Land (:04, p. 8) remarks that it is hardly to be expected that two prothallial cells will be present in one species and wholly absent in another of the same genus. The question, however, cannot be settled in this summary manner. Jaccard's observations can be set aside only after further examination of the same species he studied, and not by the examination of another species.

Miss Ferguson (:01, Pl. 12, Fig. 6) figures pollen grains of *Pinus strobus* with two prothallial cells, and also with one prothallial cell (Pl. 12, Fig. 8), and in the latter case she labels the one cell shown as the *second* prothallial cell. Of course this is on the supposition that the same number of cells is always produced in this species, and that in the pollen grain shown in her Figure 8 the first cell formed had disappeared. The question naturally arises, however, whether this may not be a real variation, and the prothallial cell shown, be the only one formed in this case. A decisive answer of course must rest on further observations.

Coker (:02) has described some interesting variations in Podocarpus. The two prothallial cells do not promptly disintegrate, but persist as cells, and the second, and sometimes the first also, behaves in a manner hitherto unknown among the Conifers, and only paralleled among the Cycads and in Ginkgo. The nuclei enlarge and become as conspicuous as the generative nucleus. The cytoplasm of the second prothallial cell loses its individuality and its nucleus slips from its former position and lies free in the general cytoplasm. This nucleus may divide amitotically before liberation. In such cases the two nuclei generally slip out in different directions and place themselves on opposite sides of the generative cell (Coker, :02, Pl. 5, Figs. 8, 10). The number of cases in which this division occurs is probably as great as the number in which it does not occur. Hundreds of cases were found. In not a few cases the first prothallial cell liberates its nucleus into the general cytoplasm.

Arnoldi (: 00) has reported a variation somewhat similar to this in *Cephalotaxus fortunei,* where he found the *tube nucleus* dividing amitotically, thus showing three free nuclei in the end of the pollen tube besides the generative cells. Arnoldi actually observed one case of division of the tube nucleus. He also reports that the nonfunctional male nucleus may divide amitotically in the upper part of the egg. Two tube nuclei were reported by Juranyi ('72, Pl. 34, Figs. 11, 12) in Ceratozamia. Two or more tube nuclei have been reported in the angiosperms, (Chamberlain, '97; Fullmer, '99; Smith, '98). The last-named author found two tube nuclei in half the pollen grains examined in *Eichhornia crassipes.*

Juel (: 04) has found in Cupressus a variation which he seems to have discovered for the first time among the gymnosperms. In the pollen tube of this genus he found not merely two sperms, but from eight to ten, or even twenty in some cases. Chamberlain ('97) had found in angiosperms occasionally a pollen grain with three sperm nuclei. In the same species, *Lilium philadelphicum,* he also found a number of cases of a prothallial cell cut off at one side of the pollen grain.

Thompson (: 05) has reported that in the pollen tube of Araucaria and Agathis there are supernumerary nuclei. His preliminary note did not disclose their origin.

Lopriori (: 05) shows that in *Araucaria bidwellii* these supernumerary nuclei arise from cells formed in the pollen grain, primarily by unequal division of the large cell of the pollen grain, and the smaller cells thus cut off may themselves divide until there are fifteen or more. These cells at first have walls but the walls dissolve and the nuclei come to lie free in the pollen tube. Lopriori believes these cells are spermatogenous cells and the nuclei derived from them male nuclei. The writer does not accept this interpretation of Lopriori and will give reasons for his opinion in connection with a later discussion.

Miyake (: 03) found in *Abies balsamea* that the second sperm nucleus, the tube nucleus, and the stalk nucleus which are left in the upper part of the egg after fertilization, may all divide, or attempt to divide, before they disintegrate. He says that the division figures are more or less abortive or abnormal, hence the divisions are apparently mitotic ones. This author saw one case of "double fertilization," in which the second sperm nucleus united with a secondary segmentation nucleus of the fertilized egg.

If one could always rely on the observations reported in the literature, the conclusion would have to be drawn that in other gymnosperms than Cupressus and possibly the Araucarineæ there were variations in the number of male cells. Tschistiakoff supposed that there might be numerous spermatozoid mother cells formed and Strasburger ('80, p. 49) writing of *Juniperus virginiana* states that one of the two primordial cells found at the tip of the pollen tube, divides into two, and often into two more, and these are used for fertilization. At a later date, however, the same author states ('92, p. 32) that in all the cases he has had opportunity to study exactly, the generative (primordial) cell of gymnosperms divides into two sister cells. Both are generative and even in the Cupressineæ both are called to perform a sexual function (p. 33). In the Abietineæ a difference in size between the cells makes its appearance, and finally in Taxus a very unequal division of the generative cell takes place, and only the larger sister cell functions in fertilization. Since that statement was written by Strasburger, numerous writers all report only two male cells from each pollen grain, and we must conclude that the earlier observations were not correct as to the larger number; so that Cupressus stands alone among the gymnosperms in forming numerous sperm cells, unless the supernumerary nuclei reported in the pollen tube of Araucarineæ by Thompson (:05) and Lopriori should prove to be sperm cells, as Lopriori believes or else this variation among the gymnosperms has been generally overlooked. The variation in the *size* of the male cells formed is common.

In a recent work on the gymnosperms, in fact the only one in which an adequate discussion of the group is given from the modern point of view (Coulter and Chamberlain, :03) a variation is noted in the Cycadaceæ and Ginkgo, as compared with other gymnosperms, in the relative position of the "stalk" and "body" cells. They are said to stand side by side, instead of dorsiventrally as they do in the other gymnosperms. These descriptions (pp. 25, 42) are evidently based on the descriptions and figures of Hirase for Ginkgo, and of Ikeno for Cycas. If Webber's completed work on Zamia (: 01) had appeared somewhat sooner the descriptions as to Ginkgo would necessarily have been different in the above-mentioned work. Webber shows very conclusively that in Zamia, and in all probability in Ginkgo also, the division into stalk and central cell (body cell) does not leave those cells side by side, but in the position found in the other groups of gymnosperms. The bulging of the last vegetative prothallial cell into the stalk cell probably led to a wrong interpretation of the earlier observations. Webber pointed out that the work of Ikeno and Hirase was obscure on this point, and that before it can be certainly concluded that any Cycad divides so as to bring the stalk and body cell side by side, except as an unusual variation, the figures showing the stages of development must be more convincing than those now available.

Lawson (: 04) found a variation in the behavior of the nuclei and cells in the pollen grain of *Cryptomeria japonica,* as compared with other gymnosperms which have the same number of nuclei. The primary spermatogenous nucleus is free in the cytoplasm of the pollen grain and not separated from the tube nucleus by any membrane, except for a short time, and even then the membrane is so difficult to distinguish that it almost escaped the observation of the author.

Variations in the pollen grain of several gymnosperms have been reported in the *Botanical Gazette* by Coker (: 04a) one of which is much like some of those found in *Picea excelsa* by the present writer. Coker's Figure 7 of *Larix europœa* is similar to my Figure 8. His Figure 6 is merely an earlier stage of the same thing. His Figure 8 of *Larix europœa* and Figure 4 of *Cupressus sempervirens* show a condition that is like that found by Lawson in *Cryptomeria japonica.* Coker's Figure 9 of *Larix europœa* shows the last division wall in the pollen grain straight across, as is usual in Cycas, instead of the strongly bulging wall that is usual in Larix.

Coker merely reports these as abnormal grains, apparently not attaching any significance to them. It seems to the writer that they are of some significance, at least as indicating the limits of variation in the different genera and species of gymnosperms, and when our knowledge of these variations is more complete they may be of some assistance in determining whether or not the structure of the pollen grain in gymnosperms is at the present time in a state of stable equilibrium or in a process of retrogressive modification. Coker's Figures 6 and 7 will be further noticed in connection with some of mine.

PICEA EXCELSA

In presenting my own observations I have considered it desirable to illustrate rather fully the different variations found in the pollen grains of *Picea excelsa* L., and these illustrations show a surprising range in the variation in internal structure of the pollen. One fact that seemed especially significant was that so few pollen grains could be found showing satisfactorily what has been called the "normal" structure, that is showing *two* disintegrating prothallial cells. For the larger number of pollen grains showed only one such disintegrating cell without a trace of a second, even as a split in the wall of the pollen grain. In order to get at the proportion of those which showed only one disintegrating prothallial cell, 466 pollen grains were counted. Only those were counted which lay in the position most favorable to the necessary observation, that is, the side view as shown in Figs. 1–6 (Pl. 1). No pollen grain was counted unless its internal structure seemed reasonably clear. Of the total of 466 grains counted, 310 or 66.5% showed only one disintegrating prothallial cell with no trace of another even as a split in the wall; 73 or 15.7% showed more or less plainly two disintegrating prothallial cells; 18 or 3.8% showed no disintegrating cells; 65 or 13.9% were doubtful as to whether they had one or two disintegrating cells. Even if all the doubtful ones are counted as "normal" the percentage of those grains which showed only one prothallial cell is still more than twice as great as the "normal." Of course it may be said that in those grains in which only one prothallial cell showed, the one first formed had completely disappeared. But if it is not to be seen, what evidence is there that it was ever formed? Two reasons may perhaps be given for believing in its formation and disappearance. First, in many cases in which two prothallial cells may be recognized the one first formed is barely recognizable as a mere slit in the wall of the pollen grain, and it is probable that in some cases the cell disappears so completely as not to be recognizable at all. Second, it is said that the pollen grain of Picea always forms "normally" two prothallial cells which disintegrate more or less, hence when only one is found the other must have disappeared. Some validity must of course be granted to the first reason given. It is probably true in some cases, but it is absolutely impossible to prove its truth in a given case, hence the number of cases in which it is true is purely a matter of conjecture. As to the second reason given, it is a conclusion based on observation, and cannot be used to prove the correctness of the observations upon which it rests. Back of it lies the assumption that the "normal" is invariable, an assumption which is far from the truth. Even if some pollen grains of Picea have been shown to form two disintegrating prothallial cells, it does not at all follow that they do so invariably.

Fig. 4 (Pl. 1) shows a pollen grain in which there were undoubtedly *three* disintegrating prothallial cells. All of them were persistent, and separate from each other, and from the antheridium part of the pollen grain, so that there was no difficulty in recognizing the number certainly. This grain was at the stage preceding the division which separates the stalk and the central cell. The third disintegrating cell cannot be interpreted as a stalk cell, first, because it so closely resembles the other disintegrating prothallial cells in size and appearance, and second, because it is so completely separated from the dome-shaped cell to which it would be closely attached if it were a stalk cell.

Fig. 5 (Pl. 1) shows a variation in the *position* of the prothallial cells, though their *number* is "normal," that is, two. In Fig. 6 (Pl. 1) there is a variation in both the number and the position. Three pollen grains were seen like Fig. 5, but only one like Fig. 6. The latter shows without any doubt whatever a case of three prothallial cells in addition to the stalk cell, central cell, and large cell that forms the pollen tube. If Fig. 4 has been correctly interpreted, it and Fig. 6 furnish direct proof that the number of prothallial cells in Picea may vary from what has been called the normal in the direction of a greater number and it seems much more probable that they would also vary in the direction of a smaller number, since so many of the gymnosperms, as Thuja and Juniperus, have no vegetative prothallial cell.

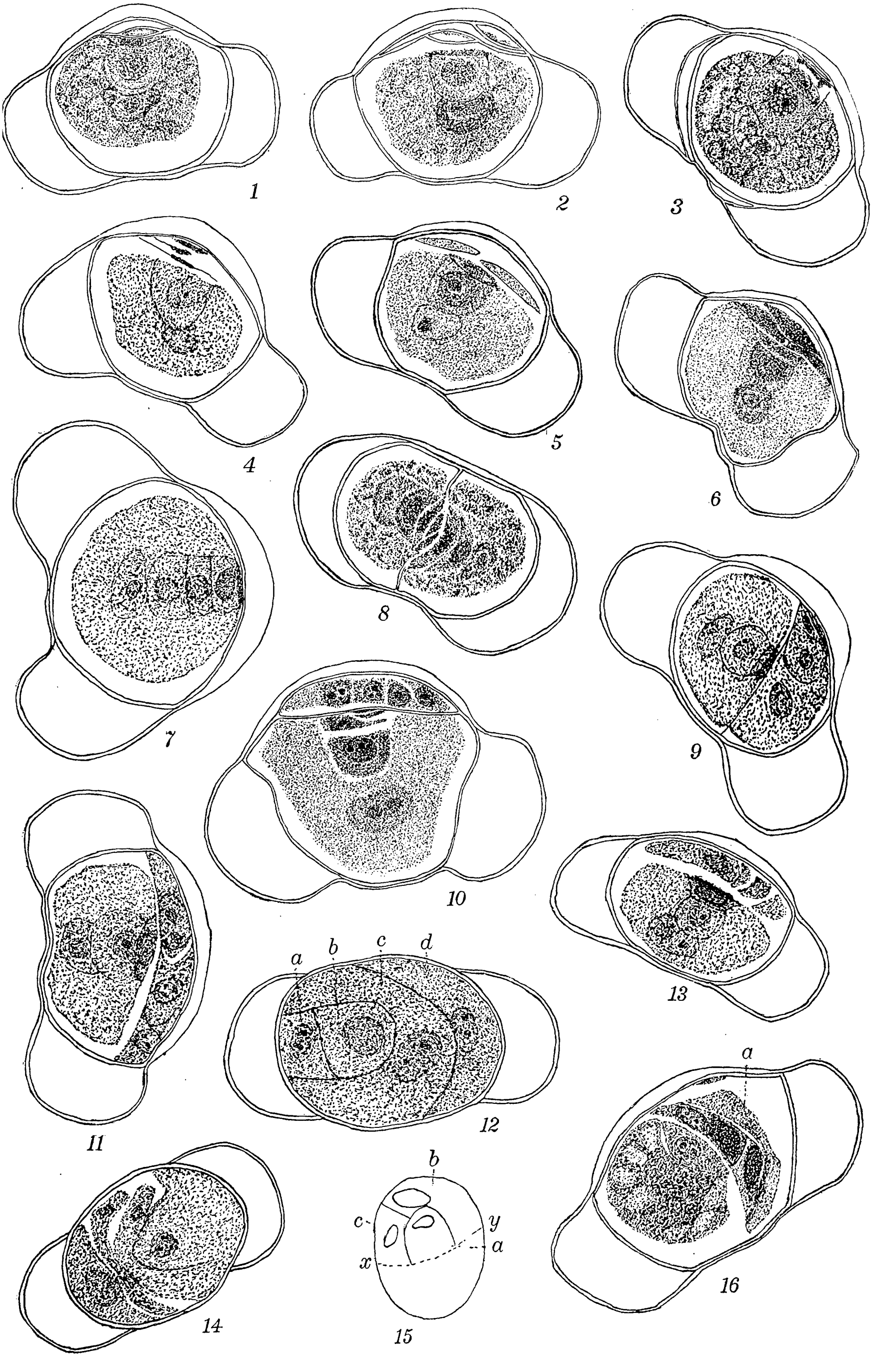

PLATE I.

Picea excelsa, variation in the pollen grains.

Fig. 2 (Pl. 1) also seems to show three prothallial cells, one with no protoplasm, and two with very little. In this case the walls of the cells had persisted after the protoplasm had almost or entirely disappeared, and so far as appearance was concerned seemed to be like the inner layer of the pollen grain wall and continuous with it. Usually, however, these cells do not show so persistent and evident a membrane. Fig. 1 has the number regarded as the normal for Picea. In this case also the membrane persisted after the protoplasm was entirely gone in the first one formed, showing that the membranes were not plasmic, but in all probability of cellulose. No microchemical tests were made on them. Fig. 1 shows a distinct separation of the protoplasm of the central cell from that of the large tube cell. This separation was the result of plasmolysis, probably due to the killing fluid. The boundary of this cell, however, appeared to be only a plasmic membrane. At least it was exceedingly thin and closely applied to the surface of the protoplasm.

Fig. 3 (Pl. 1) shows the typical arrangement of cells, with two prothallial cells, but between the main part of the pollen grain and the wings there are two spaces cut off by cellulose walls, containing no trace of protoplasm. The writer was unable to decide whether these should be considered as cells or not. They seemed to bear no definite relation to the main part of the pollen grain. In this grain the membrane surrounding the central cell, and continued around the stalk cell, plainly projected beyond the limits of the protoplasm where it had been pulled away from the wall of the pollen grain by plasmolysis. This membrane was very much thinner than the inner cellulose layer of the wall of the pollen grain, but if it was only a plasmic membrane it was at least much firmer than the protoplasm which it bounded, and from which it was partly separated by plasmolysis.

There is shown in Fig. 7 (Pl. 1) a variation somewhat different from those already discussed. Only one disintegrating prothallial cell is apparent, but projecting into the tube cell of the pollen grain is a row of three cells of approximately equal size and structure except that the innermost one of the row is dome-shaped while the others are flattened against the adjacent cells. These three cells have equally large, prominent, and well preserved nuclei, and apparently either the stalk cell or the central cell of the typical pollen grain has in this case divided again. There was no indication as to which of the two furnished the third cell found here. Another interpretation of this pollen grain is possible. The outermost of the three almost equal cells might be regarded as the second prothallial cell. The writer does not accept this interpretation of that cell because of its large size, its prominent and well preserved nucleus, and its close connection with and general resemblance to the adjacent cell.

A very different type of variation is illustrated in Fig. 8 (Pl. 1). Here the pollen grain, while having the typical form externally, is divided into two equal portions by a wall perpendicular to the long axis of the grain. This wall was very plain where it joined the outer wall of the grain, but where the protoplasm lay against it on both sides it could not be recognized certainly, and it was represented in the drawing only where is was unmistakable. Its position is probably indicated by the cleft in the protoplasm which throws five cells on one side and three on the other. This gives the typical form and arrangement of cells with two prothallial cells on one side, while on the other side of the dividing wall no prothallial cells are formed, and the three cells present apparently represent a stalk cell, a central cell, and a tube cell. The writer believes this difference in the two halves to be of some importance in relation to the variation in number of prothallial cells. If the number of those that disintegrate is invariably two for each pollen grain, which ordinarily has one antheridium, in this double grain where there are two antheridia, there ought to be two disintegrating cells for each antheridium. If it be supposed that one set of two prothallial cells serves as the basis of two antheridia, variation from this arrangement and number of prothallial cells is found in other grains of this same type, containing two antheridia. Fig. 9 shows a grain with the two antheridia in different positions in their respective portions, and each has only one disintegrating prothallial cell visible. Thirty-two grains of this double type were studied in more or less detail. In most of them the two antheridia were placed as in Fig. 8. There was much variation in the number of the prothallial cells connected to the various antheridia. Put in the simplest form and using the single antheridium instead of the double pollen grain as the basis of comparison the following table shows the amount of variation.

	2 Pr. Cells	1 Pr. Cells	No Pr. Cells	Cells Uncertain	Total Antheridia	Total No. Pollen Grains
No. of antheridia	4	24	18	18	64	32

In the group marked uncertain, the uncertainty was sometimes due to inability to recognize clearly the total number of cells present, and in other cases the position of the wall dividing the pollen grain was uncertain where it ran between the disintegrating prothallial cells. Only one sixteenth of this lot of antheridia shows what has been called the normal number of prothallial cells. Of even more importance than this fact is the further one that in no case did both the antheridia in the same double grain have two prothallial cells, as should have been the case if two is the normal number. There were several cases in which the numbers for the two antheridia were respectively 2 and 1, 2 and 0, 1 and 1, 1 and 0, 0 and 0. In those cases in which there were two prothallial cells in one part, and one or none in the other part of the double grain, it cannot be argued that some had disappeared by disintegration, because the two parts of a double grain are usually at about the same stage of development, and there is no reason to suppose that two cells should so completely disappear in one part, and none in the other. At least some cases should have been found in which both parts of the double grain had two prothallial cells, and most of the double grains should have shown the same number of prothallial cells on the two sides of the dividing wall. Since neither of these conditions was found to exist in the grains examined, and in the light of other facts reported in earlier paragraphs of this article, the writer feels justified in drawing the following conclusions: —

1. The number of disintegrating prothallial cells in the pollen grain of *Picea excelsa* L. is not invariably two, and when a grain is found which shows only one such cell it is not necessarily the *second* prothallial cell and should not be so named. At the most it can only be said that it is uncertain whether the case is one in which a prothallial cell has completely disappeared, or whether it is a case of *normal variation.*

2. The number of vegetative prothallial cells certainly varies from one to three, though the latter number is rare. It may also happen that there are none at all. This condition is comparable with that found in those gymnosperms which typically produce no prothallial cell, as Juniperus, Taxus, and others. In Picea it was found in 3.8 % of the pollen grains that contained only one antheridium.

3. The prothallial cells may vary in position as well as in number. The usual arrangement is dorsiventral, forming a single continuous row with the stalk and central cells, but two prothallial cells may be placed laterally in relation to each other instead of dorsiventrally, and in case of three cells the arrangement may be partly lateral and partly dorsiventral (Pl. 1, Figs. 5, 6).

4. In very rare cases the variation in number may extend to that part of the row of cells which usually is composed of the stalk cell and the central cell. An extra cell may be formed at this point perhaps by division from the central cell. The stalk is then two cells high.

5. The number of prothallial cells formed in the majority of pollen grains would seem to be *one*, if the results obtained in counting are to be fully relied upon, since 66.5 % of them showed but one prothallial cell as against 15.7 % which showed two. This difference is probably greater than it should be, since it cannot be denied that one cell *may* have disappeared in some of those grains which showed only one.

6. The membranes of the prothallial cells sometimes persist after the complete disappearance of the protoplasm, hence they cannot be plasmic, but are probably cellulose. In other cases there is no trace of cellulose walls.

Coker (:04a) interpreted his double pollen grains of Larix shown in his Figures 6 and 7, as having been formed by one instead of two divisions of the pollen mother-cell, and the daughter cells having been retained within the mother-cell wall. In the pollen grains of Picea studied by the writer this interpretation seems very improbable, first, because no trace of the mother-cell wall was observed around the pollen grain; second, because of the fact that the "wings" of the pollen grain were always present, and always typical in number and arrangement. If these double grains were contained in a pollen mother-cell, the pollen mother-cell wall was cuticularized and expanded into two wings. No indications of this condition were observed.

A pollen grain with a very different appearance from that of any yet described is represented in Fig. 10 (Pl. 1), which is a drawing of the pollen grain whose discovery led to the observations recorded in this article. In this grain we have the typical tube cell, central cell, stalk cell, and what seem to be two disintegrating prothallial cells, but in addition we find lying along the dorsal wall of the pollen grain a row of four rather prominent cells, each with a nucleus. These cells appear to be of unequal size and of somewhat different shapes. They are separated from the rest of the pollen grain by a wall which was continuous with the inner layer of the wall of the grain, and which appeared just like it in structure. The presence of this wall was made evident by the contracted state of the protoplasm adjacent to it.

The structure of this grain was reported at the meeting of botanists mentioned above, and it was suggested that possibly the four cells represented a prothallium or gametophyte of a much larger size than usual, and the pollen grain showed a reversion to an earlier type of structure. At that time the writer saw no way of determining definitely whether that was the correct interpretation of the structure discovered. In succeeding years, it was found that the class material from which that pollen grain came also contained numerous others similar to it as well as the variations of the different kinds that are reported in this article.

After studying a number of grains of this same type the thought occurred that perhaps a clue to the meaning of the unusual structure might be obtained if the same grains could be studied from the dorsal as well as from the lateral view. Since the pollen grains were mounted in clove oil, this could be done, by causing them to roll over while observing them under the microscope. By repeated trials it was found possible to study some of these abnormal grains from all possible points of view, dorsal, ventral, lateral, and end views. The dorsal and lateral views were the most important.

A total of 22 grains of this structure was studied, and many of them were drawn in two or more views for purposes of comparison. Figs. 11 and 12 (Pl. 1) are respectively the lateral and dorsal views of the same pollen grain, and Figs. 13 and 14 (Pl. 1) are the corresponding views of another grain. A study of the structure shown in Figs. 12 and 14 as well as others more or less similar to them finally suggested what the writer believes is the correct interpretation of the extra cells along the dorsal wall of pollen grains as shown in Figs. 10, 11, and 13. In Fig. 12 it will be noticed at once that the cells labeled *a* and *b* are like the stalk and central cell in a typical pollen grain, while cell *c* has the same relation to the others as a tube cell, and cell *d* has no corresponding part in the typical pollen grain. No cells appear to correspond to the prothallial cells in the typical grain. In Fig. 14 the correspondence to the typical pollen grain is less clear than in Fig. 12. Nevertheless there is a distinct suggestion of a similar structure. In short, a study of the dorsal view of as many of these grains as possible led to the conclusion that grains of this type are not so very different from the type shown in Fig. 8. They are double pollen grains, but the division wall, instead of running from dorsal to ventral side and dividing the grain into two equal parts, divides it into two very *unequal* parts, *and cuts off the smaller portion along the dorsal wall.* This interpretation was made more certain on looking over all the rough drawings made during the study, when it was found that there was a very complete series of transitional stages between the structure shown in Fig. 8 and that in Fig. 12. In these transitional stages the division wall ran from some point on the dorsal side obliquely toward one of the wings. Fig. 9 is an example of one of these transitional stages, and Fig. 15 is a diagram of what was seen in a dorsal view of a pollen grain with an oblique division wall. The dotted line *xy* is presumably the line of junction of the oblique division wall with the dorsal wall of the grain. This was of course not plain, owing to the oblique position of the receding wall. Cell *a* bulges into cells *b* and *c* like the cell which divides to form the stalk and central cell. Cells *b* and *c* taken together represent the tube cell. If, as is generally supposed, the tube cell represents an antheridium wall, then we have here a case where the antheridium wall is two-celled, a very unusual thing among the gymnosperms. One other case was observed almost exactly like this one, except for a slightly different position of the wall separating cells *b* and *c*. It is just possible that the two tube nuclei found by Juranyi ('72) in the young pollen tube of *Ceratozamia longifolia* and by Arnoldi (:00) in *Cephalotaxus fortunei* represent two cells in the antheridium wall or tube cell of the pollen grain, but that interpretation can hardly be insisted upon very strongly on the evidence now available.

In the case of the two or more tube nuclei in the pollen tube of angiosperms, the above interpretation ought to be considered, but here there will be even more hesitation over its acceptance than in the gymnosperms.

As regards the large number of nuclei found in the pollen tube of Agathis and Araucaria, Lopriori concludes that in *Araucaria bidwellii* they are male nuclei, and that the cell-complex which is formed in the pollen tube, and from which they are derived, is a spermatogenous cell-complex. Lopriori's conclusion is evidently much influenced by Juel's discovery of the numerous male cells in Cupressus. The present writer cannot accept Lopriori's conclusion until there is direct evidence that the nuclei in question do actually function in fertilization. The reasons for rejecting the conclusion at the present time are these: —

1. The cell-complex from which the nuclei are derived is formed in the pollen grain and not in the pollen tube as Juel found in *Cupressus goweniana*.
2. It is formed, not from a single generative cell, but primarily by the successive unequal divisions of the large cell of the pollen grain, though secondarily the small cells thus formed may themselves divide in different directions.

These reasons seem sufficient to show that the cell-complex is not spermatogenous in its nature, but that it is the vegetative part of a male gametophyte, and as such is exactly the condition which the present writer thought he had found when he first saw the pollen grain of Picea represented in Fig. 10 (Pl. 1). Though this interpretation proved to be wrong for Picea, Thompson and Lopriori have probably discovered in the Araucarineæ a group of gymnosperms which have the multicellular male gametophyte, and in this particular at least they show the most primitive condition of any of the gymnosperms.

It is to be hoped that these investigators will follow out the full life history of all the cells and nuclei found in the pollen grain and tube of the Araucarineæ.

In a recent short review of Lopriori's paper, C. J. Chamberlain also rejects that writer's conclusion that the numerous nuclei are male nuclei, but he gives no reasons therefor except "judging by his figures."

The structure in Fig. 16 (Pl. 1) requires little discussion. It represents one of the somewhat irregular variations in a double grain, but the portion marked *a* seems to consist of a mass of protoplasm completely cut off from the rest, and contains no trace of a nucleus. In order to determine the frequency with which the variation of the double pollen grain occurred, a count was undertaken. Fresh drops of clove oil containing the pollen grains were mounted and all the grains on the slide were systematically counted. A separate list was made of all the grains that were double, both those like Fig. 10 and like Fig. 8 and also the ones intermediate between these two. The total number counted was 1120. Of these there were 27, or 2.4 % which showed the double character more or less plainly. This was a much higher percentage than was expected, but there is no reason to think a mistake was made in the counting. Indeed it is more probable that some of the double grains may have been overlooked. If any of them lay in the position with the dorsal or ventral side toward the observer they would almost certainly have been overlooked since in that position their double nature is very difficult to recognize. The proportion among those that were counted did not impress the observer as being any greater than the proportion among the hundreds that were not counted but were looked over in making the study of the variations found.

As a result of the study of the type of variation shown in Figs. 10–14, the following additional conclusions may be drawn: —

1. The three or four cells lying along the dorsal side of the pollen grain of this type do not constitute a prothallium or gametophyte of unusual size, an interpretation which the writer at one time thought a possibility.

2. They constitute the smaller portion of a pollen grain separated by a division wall into two very unequal portions, each of which may form a more or less typical antheridium.

3. The cells of the larger part of such a pollen grain are similar to the cells in a typical single pollen grain, and cases were observed in which the number of prothallial cells visible was 2, 1, and 0.

4. In the smaller portion of such a double pollen grain there is more variation in the size, number, and arrangement of cells, but generally there is a distinct resemblance to the typical antheridium. In several cases the similarity was striking and unmistakable.

5. In two cases where a cell which is like the one that divides into the stalk and central cell, was plainly marked, the rest of that portion of the pollen grain, which corresponds to the tube cell or antheridium wall, was divided into two cells by an anticlinal wall, that is, the antheridium wall was two celled (Pl. 1, Fig. 15).

6. In the material examined the proportion of double pollen grains was found to be 2.4 % in a count of 1120.

Writers who feel justified in labeling a single prothallial cell as the *second* one, may be inclined to reject some of the conclusions reached in this article, especially the conclusion that a majority of pollen grains in *Picea excelsa* form only one prothallial cell (p. 271) on the ground that so much of the material studied was abnormal. It has already been admitted that there is room for argument as to the *number* of pollen grains in which only one prothallial cell is formed, and the point at issue is really the determination of what is the normal number, or to state it more clearly perhaps, the problem is to determine the limits of variation in the number of cells in the pollen grains of Picea, and the relative frequency of the different numbers. Stated in that form, it is apparent that the abnormal, which is only the less frequent, is as much a part of the problem as the normal. The abnormal is only normal variation. Hence conclusions cannot be rejected because based on observation of abnormal structures.

After consideration of both the historical and original parts of this article it is obvious that variation in the number and arrangement of the cells found in the pollen grain and pollen tube is widespread among the families, genera, and species of gymnosperms. They occur in those species which usually have no disintegrating prothallial cells, as well as in those which have one or more such cells. These variations cannot be dismissed with the statement that they are abnormal, since the abnormal is only the less frequent modification. It seems to the writer that they may have a significance beyond the mere fact that the individual species vary.

A comparison between angiosperms and gymnosperms as regards the structure of the pollen grain recalls the well known difference. The essential structure of the pollen is much more uniform among the angiosperms, showing a spermatogenous cell usually free within the larger tube-forming cell, the whole structure to be regarded as probably a gametophyte reduced to an antheridium, the tube cell forming the antheridium wall, and generally there are no prothallial cells. Among the gymnosperms the structure of the pollen grain is not uniform in the different families and genera, and it is not so much reduced as in the angiosperms. In the more complex of the gymnosperms (Picea for example) there is not only an antheridium represented, but there may be from one to three disintegrating prothallial cells, and in addition the antheridium has a cell, the so called stalk cell, which has nothing to correspond to it in the angiosperms. This stalk cell is a sister cell to the so called body cell or central cell, which divides to form (usually) two sperm cells, and therefore corresponds to the spermatogenous cell in the angiosperms. In those gymnosperms with the simpler pollen grains no disintegrating prothallial cells are formed, but the stalk and central cells are uniformly present, the mother cell of these two being cut off at one side of the large tube-forming cell. In the pollen of the simplest gymnosperms therefore there is one cell more than in that of the angiosperms, that is the stalk cell. Cryptomeria seems nearer to the angiosperms in this particular than any other gymnosperm, since Lawson (:04) has found that the cell cut off at the side of the pollen grain is soon set free and lies within the cytoplasm of the large tube-forming cell. Chamberlain ('97) has also found cases in pollen grains of angiosperms which show one prothallial cell and more often it is found in angiosperm pollen that the spermatogenous cell is cut off at one side of the grain instead of lying free in the tube cell (Coulter and Chamberlain, :03, pp. 134–135).

The gymnosperms then, compose a group containing various transitional stages, as regards the pollen grain structure, between the very reduced condition characteristic of the angiosperms and the condition found among the pteridophytes in which the male gametophyte is more or less developed. Indeed some pteridophytes as Isoetes and Selaginella have more reduced male gametophytes than some gymnosperms, as Picea and Pinus.

The facts just presented suggest the question whether the gymnosperms at the present time are in a process of retrogressive modification in the direction of the condition found in the pollen of the angiosperms, or have the gymnosperms reached a condition of stable equilibrium, not so much reduced as that of the angiosperms and with different groups of genera stable at different stages in the reduction process.

The mental attitude of those investigators who insist on labeling a single prothallial cell as the second one implies a stable condition, with the number of cells formed invariable, and this mental attitude seems to be the common one among the writers on the subject. It seems to the present writer, however, that the number and wide distribution of the variations found among the gymnosperms may indicate that the gymnosperms, or at least some of them, are not in a state of stable equilibrium as regards the structure of the pollen grain, but that the process of reduction of the male gametophyte is in progress. In Picea, for example, a very few cases were found in which three vegetative prothallial cells were seen, 15.7 % of the grains counted showed two, 66.5 % showed one, and 3.8 % showed none. The number in others was uncertain. In the case of the double pollen grains divided into nearly equal parts, several showed no prothallial cell in one part, and a few showed none in either part. The extreme limits of variation in the number of prothallial cells in the pollen grain of *Picea excelsa* are therefore from 0 to 3, with 1 as the number of greatest frequency.

These facts do not *prove* that Picea is in a condition of progressive reduction of the male gametophyte, but at the very least they allow that interpretation, and it is set forth merely tentatively. Other investigations may either confirm or disprove it. Should further investigations confirm this conclusion we should have a case of a structure changed by gradual modification rather than by mutation. The occasional variations found in angiosperms in which the pollen grain shows a vegetative prothallial cell, or several tube nuclei, or the spermatogenous cell cut off at one side of the grain instead of lying free in the cytoplasm of the tube-cell (Coulter and Chamberlain, :03, p. 134–135) may possibly have a similar significance. If so, the condition now found in the pollen of angiosperms also has come about by gradual modification, and the variations mentioned show that the "normal" structure is not so firmly fixed but that a small proportion of the grains produced reverts to a condition which was a stage in the gradual development. If this interpretation of the variations is rejected, then they must be looked upon as mutations, for whose cause we have nothing at all to suggest.

It is worth noting that among the gymnosperms the reduction of one structure in the pollen grain is not necessarily accompanied by the reduction of other structures in the same pollen grains. For example in Cupressus (Coker, : 04) no sterile prothallial cell is cut off, and yet many sperm cells are formed instead of only two (Juel, : 04). On the other hand, Picea, which may have as many as three sterile prothallial cells not only has the sperms reduced to two nuclei in one cell but even one of those nuclei has undergone a further reduction in size and only one, the larger, is functional (Miyake, : 03). In the reduction of the sperm cells therefore, some of the gymnosperms have been modified to even a greater extent than the angiosperms. The degree of reduction seems to be closely associated with "use and disuse" of the parts concerned. Reduction of one of the sperm cells takes place only in cases where both could not function in fertilizing the egg. In the angiosperms the second sperm cell functions by uniting with the polar nuclei to form the endosperm, and this functioning whether a true fertilization or not, has been sufficient to prevent the reduction and possible suppression of one of the sperm cells.

The observations of Miyake (: 03) that in *Abies balsamea* the second sperm nucleus may divide, or attempt to divide before it disintegrates in the upper part of the egg, and especially the observation that in one case this second sperm nucleus united with one of the second segmentation nuclei of the fertilized egg, is suggestive as to the possible way in which "double fertilization" may have arisen. The sperm nucleus that is left over after the union of the other one with the egg nucleus, has a tendency to unite with any nucleus that may be available, and in the angiosperms the polar nuclei offer the opportunity for such a union. It is not at all necessary that either of the polar nuclei be the homologue of an egg as Bonnier (: 05) believes, in order to explain the union with the male nucleus, since the latter has been shown to be able to unite with a purely vegetative nucleus, even of the sporophytic generation. This observation of Miyake's seems therefore to strengthen the evidence that the endosperm of angiosperms was vegetative phylogenetically (Coulter and Chamberlain, :03, p. 183), and to weaken correspondingly the view that it was strictly sexual in its origin (Bonnier, :05) and that the endosperm is to be regarded as a modified embryo.

If the introduction of the characters of the male parent into the endosperm modified that endosperm so as to make it produce food more suitable to the embryo with characters inherited from the same male parent (Sargent, :00, p. 708) then the embryo would be more vigorous, and more fit to survive in the struggle for existence than the one not nourished by such an endosperm Hence those individual plants in which "double fertilization" took place would finally become dominant in the competition with others of the same species.

The reduction, disintegration, and final complete suppression of the vegetative part of the male gametophyte may also depend on the law of "use and disuse." The vegetative cells of a well developed male gametophyte function chiefly in the process of photosynthesis. In most of the gymnosperms this function is impossible since the pollen grain is soon enclosed in the micropyle and light is excluded by the thick scales of the fertile cone. It is then a waste of energy and food materials to form cells that do not function, and this waste is only in part compensated for, if the cells formed disintegrate later and thus become available as food for the work necessary in forming sperms. Disuse leads to disintegration of the useless cells, and their complete suppression is the most advantageous condition of all.

It is interesting to note in this connection that in Araucaria which probably has the well developed male gametophyte, the pollen grain does not enter the micropyle (Thompson, :05), but germinates at the distal end of the ligule, more or less entangled on its serrated edge. From this point the pollen tubes pass in the grooves on the surface of the ligule or scale, a distance of an inch or more, to the micropyle. It would seem from this description of Thompson's that the pollen grains are exposed to the light during the development of the tubes, hence the multicellular male gametophyte present may function in photosynthesis. Continued use has prevented in this case the reduction which took place in those species in which the pollen grain enters the micropyle directly.

In addition to the specific conclusions in regard to Picea (see pp. 270 and 275) the following more general ones are drawn, some of which are suggested rather than demonstrated:—

1. The variations in the male gametophyte and other structures in the pollen of gymnosperms, indicate that in this group there are examples of progressive reduction of some of those structures.
2. This reduction is by a gradual modification and not by mutation, and is due to the disuse of the vanishing structures.
3. The different structures are not necessarily reduced to a corresponding degree in the same species.
4. The suppression of the male gametophyte in gymnosperms is a distinct advantage where the function of photosynthesis is impossible to it.
5. The occasional prothallial cell in the pollen of angiosperms indicates that in this group also there was a reduction by gradual modification.
6. The division of the non-functional male nucleus in the egg of some of the gymnosperms and its fusion with a segmentation nucleus of the fertilized egg in *Abies balsamea,* strengthens the evidence for the view that the endosperm of angiosperms is phylogenetically a modified gametophyte and not a modified embryo.
7. The extremes in the variation of the male gametophyte among the genera of gymnosperms are found in Araucaria with a gametophyte of from 20 to 44 cells, (provided my interpretation of Lopriori's observation is correct) and *Cryptomeria japonica* (Lawson, :04), in which there is not only no vegetative male prothallium, but in which even the division wall between the tube nucleus and the primary spermatogenous nucleus is so fugitive as almost to escape detection.

The writer has no intention of making any further investigations along this line, as his special interests lie in another direction, but he felt that the opportunity to make the observations here recorded, though arising only incidentally, ought not to be neglected.

Botanical Department of the
University of Michigan

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